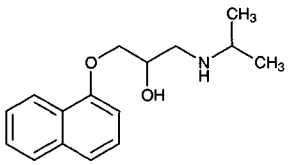
butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Propranolol



Molecular formula: $C_{16}H_{21}NO_2$
Molecular weight: 259.35
CAS Registry No.: 525-66-6, 318-98-9 (HCl)
Merck Index: 8025
Lednicer No.: 1 117; 2 105, 212

SAMPLE

Matrix: bile, perfusate
Sample preparation: 500 μL Perfusate or 100 μL bile + 50 μL 50 μg/mL labetalol + 1 mL 1 M pH 10.3 carbonate buffer + 5 mL acid-washed diethyl ether, vortex, centrifuge. Remove the organic layer and add it to 125 μL 0.5% phosphoric acid, extract, inject a 10 μL aliquot of the aqueous layer. (Deconjugate 500 μL perfusate with 250 μL 8000 U/mL β-D-glucuronidase/aryl sulfatase in 200 mM pH 4.5 sodium acetate buffer, heat at 40° for 1 h, proceed as above.)

HPLC VARIABLES

Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.6 with concentrated phosphoric acid
Flow rate: 3
Injection volume: 10
Detector: F ex 295 em 360

CHROMATOGRAM

Retention time: 5.5
Internal standard: labetalol (6.9)
Limit of quantitation: 62.5 ng/mL

KEY WORDS

sheep; liver; pharmacokinetics

REFERENCE

Ring,J.A.; Ghabrial,H.; Ching,M.S.; Shulkes,A.; Smallwood,R.A.; Morgan,D.J. Fetal hepatic propranolol metabolism. Studies in the isolated perfused fetal sheep liver, *Drug Metab.Dispos.*, **1995**, *23*, 190–196.

SAMPLE

Matrix: blood
Sample preparation: Prepare a silica SPE cartridge. Fill 3 mL cartridge with 500 mg Silica gel 60 (Merck). Condition it with 2.5 mL MeOH and with 2.5 mL water. Add 500 μL plasma or serum to the SPE cartridge. Wash with 1 mL water, elute with 3 mL MeOH (added dropwise).

Evaporate eluates to dryness under a gentle stream of nitrogen. Reconstitute residue in 200 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Keystone ODS2
Mobile phase: MeCN:tetrahydrofuran:50 mM pH 5.00 phosphate buffer 24:1:75
Flow rate: 1
Injection volume: 30
Detector: UV 204

CHROMATOGRAM
Retention time: 21
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: clindamycin

KEY WORDS
plasma; serum; SPE; propranolol is IS

REFERENCE
Liu,C.-M.; Chen,Y.-K.; Yang,T.-H.; Hsieh,S.-Y.; Hung,M.-H.; Lin,E.T. High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections, *J.Chromatogr.B*, **1997**, *696*, 298–302.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma or serum at 11300 g for 7 min, inject a 200 μL aliquot onto column A, elute to waste with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase B, after 6 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Reequilibrate column A with mobile phase A for 5 min.)

HPLC VARIABLES
Column: A 20 × 4.0 BioTrap 500 C18 (ChromTech); B 10 × 4.0 5 μm Hypersil Elite C18 + 150 × 4.6 5 μm Hypersil Elite C18
Mobile phase: A 2-Propanol:20 mM pH 7.0 sodium phosphate buffer containing 5 mM sodium octanesulfonic acid 4:96; B MeCN:116 mM pH 2.8 sodium phosphate buffer containing 2 mM sodium octanesulfonic acid 33:67
Flow rate: A 0.8; B 1
Injection volume: 500
Detector: F ex 220 em 340

CHROMATOGRAM
Retention time: 16.5

KEY WORDS
plasma; serum; column-switching

REFERENCE
Hermansson,J.; Grahn,A.; Hermansson,I. Direct injection of large volumes of plasma/serum on a new biocompatible extraction column for the determination of atenolol, propranolol and ibuprofen. Mechanisms for the improvement of chromatographic performance, *J.Chromatogr.A*, **1998**, *797*, 251–263.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg ENVI-18 (Supelco) SPE cartridge with 2 mL MeOH and 2 mL water. Add 1 ml plasma to the SPE cartridge, wash with 5 mL water and 1 mL MeOH, dry under vacuum for 10 min, elute with 2 mL 700 mM ammonium hydroxide in MeOH, evaporate to dryness under a stream of air. Reconstitute the residue in 100 μL mobile

phase and 100 μL hexane, vortex for 1 min, centrifuge at 1800 g for 5 min, inject a 20 μL aliquot of the lower phase.

HPLC VARIABLES
Guard column: 4 × 4 RP-8 endcapped (Merck)
Column: 250 × 4.6 10 μm Chiracel OD-R
Mobile phase: MeCN:250 mM sodium perchlorate adjusted to pH 4.0 with perchloric acid 40:60
Flow rate: 0.7
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 9, 12 (enantiomers)
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: propafenone
Simultaneous: amitriptyline, atenolol, bromazepam, clobazam, clonazepam, dexamethasone, diazepam, diclofenac, diltiazem, flunitrazepam, haloperidol, imipramine, lidocaine, mebendazole, metoprolol, phenytoin, praziquantel, procainamide, propoxyphene, salicylic acid, triazolam, trimethoprim, warfarin
Noninterfering: acetaminophen, albendazole, albuterol, alprazolam, cimetidine, disopyramide, fenfluramine, mexiletine, phenobarbital, pindolol, primidone
Interfering: carbamazepine, flurazepam, lorazepam, oxyphenbutazone, verapamil

KEY WORDS
plasma; chiral; SPE; propranolol is IS

REFERENCE
de Gaitani,C.M.; Lanchote,V.L.; Bonato,P.S. Enantioselective analysis of propafenone in plasma using a polysaccharide-based chiral stationary phase under reversed-phase conditions, *J.Chromatogr.B*, **1998**, *708*, 177–183.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 60 μL 1 μg/mL IS in MeCN:water 50:50 + 500 μL 1 M pH 10 potassium phosphate, vortex for 1 min, add 6 mL dichloromethane, rotate for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen. Reconstitute with 1 mL dry dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL dimethoxypropane, evaporate to dryness under a stream of nitrogen. Add 150 μL 100 μg/mL 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate in MeCN to the residue, vortex for 1 min, let stand overnight. Evaporate the reaction mixture to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN:water 50:50, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Altex Ultrasphere C18
Mobile phase: MeCN:water:phosphoric acid:triethylamine 58:42:0.1:0.06
Flow rate: 1
Injection volume: 70
Detector: F ex 280 em 340

CHROMATOGRAM
Retention time: 9 (S-(-)), 10.5 (R-(+))
Internal standard: 4-methylpropranolol hydrochloride (14.65)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (F ex 325 em 400)

KEY WORDS
plasma; human; chiral; pharmacokinetics; derivatization

REFERENCE
Wu,S.T.; Chang,Y.P.; Gee,W.L.; Benet,L.Z.; Lin,E.T. Stereoselective high-performance liquid chromatography determination of propranolol and 4-hydroxypropranolol in human plasma after pre-column derivatization, *J.Chromatogr.B*, **1997**, *692*, 133–140.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 4 μg/mL labetalol in water + 100 μL 20% sodium metabisulfite (freshly prepared) + 1 mL 1 M pH 10.2 sodium carbonate + 8 mL ether, shake gently for 10 min on a reciprocating shaker, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, centrifuge for 4 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 10 mM potassium phosphate adjusted to pH 3.4 with 5 M HCl.)
Injection volume: 50
Detector: F ex 295 em 360

CHROMATOGRAM
Retention time: 6
Internal standard: labetalol (F ex 310 em 380 (filter)) (4.8)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-hydroxypropranolol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Drummer,O.H.; McNeil,J.; Pritchard,E.; Louis,W.J. Combined high-performance liquid chromatographic procedure for measuring 4-hydroxypropranolol and propranolol in plasma: Pharmacokinetic measurements following conventional and slow-release propranolol administration, *J.Pharm.Sci.*, **1981**, *70*, 1030–1032.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 25 mg/mL ascorbic acid in water (prepare fresh daily) + 500 μL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 μL aliquot. (Buffer was prepared by mixing saturated sodium carbonate and saturated sodium bicarbonate to pH 9.4.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeOH:THF:buffer 60:6:34 (Buffer was 0.8 g ammonium acetate in 340 mL water, pH adjusted to 5.5 with acetic acid (if necessary).)
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 275 (slit width 6 nm) em 324 (slit width 10 nm)

CHROMATOGRAM
Retention time: 8
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: penbutolol
Simultaneous: metoprolol, pergolide, physostigmine

Noninterfering: acetaminophen, aspirin, atenolol, bromocriptine, chloroquine, doxorubicin, hydrochlorothiazide, indomethacin, 17-methyltestosterone, nadolol, nandrolone, practolol, quinidine, salicyluric acid, sulfadiazine, sulfamerazine, sulfamethazine, timolol, triamterene, vinzolidine, warfarin
Interfering: carazolol

KEY WORDS
propranolol is IS; plasma

REFERENCE
Miner,D.J.; Binkley,D.A.; Bechtol,L.D. Liquid-chromatographic determination of penbutolol and its principal metabolites in plasma and urine, *Clin.Chem.*, **1984**, *30*, 717–723.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 100 μL 20 μg/mL pronethalol in MeOH + 1 mL pH 10 carbonate buffer + 10 mL diethyl ether, shake for 10 min, centrifuge. Remove the ether layer and cool it to 0°, add 10 μL 12.5% phosgene in toluene, vortex for 30 s, centrifuge. Remove the ether layer and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 50 μL dichloromethane, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Pirkle Type 1-A with gamma-aminopropyl packing modified with (R)-N-(3,5-dinitrobenzoyl)phenylglycine (Regis)
Mobile phase: Hexane:isopropanol:MeCN 97:3:1
Column temperature: 20
Flow rate: 2
Injection volume: 50
Detector: F ex 290 em 335

CHROMATOGRAM
Retention time: k' 57 (S), k' 62 (R)
Internal standard: pronethalol (k' 16)
Limit of quantitation: 0.5 ng/mL

KEY WORDS
whole blood; derivatization; chiral

REFERENCE
Wainer,I.W.; Doyle,T.D.; Donn,K.H.; Powell,J.R. The direct enantiomeric determination of (-) and (+)-propranolol in human serum by high-performance liquid chromatography on a chiral stationary phase, *J.Chromatogr.*, **1984**, *306*, 405–411.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma or 0.5 mL plasma water + 50 μL 120 μg/mL penbutolol in EtOH + 1 mL 1 M NaOH + 12 mL n-heptane:isoamyl alcohol 98.5:1.5, shake mechanically for 10 min, centrifuge at 1680 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hitachi Gel 3013 spherical styrene-divinylbenzene
Mobile phase: EtOH:buffer 65:35 (Buffer was 20 mM pH 2.0 perchloric acid/sodium perchlorate.)
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: F ex 285 em 340

CHROMATOGRAM
Retention time: 14
Internal standard: penbutolol (18)

Limit of quantitation: 2 ng/mL (plasma water), 1 ng/mL (plasma)

OTHER SUBSTANCES
Simultaneous: quinidine, reserpine
Noninterfering: allopurinol, benzbromarone, diazepam, digoxin, diltiazem, dipyridamole, disopyramide, furosemide, isosorbide dinitrate, maprotiline, nifedipine, nitrazepam, trichlormethiazide, verapamil

KEY WORDS
plasma; plasma water; pharmacokinetics

REFERENCE
Yamamura,Y.; Uchino,K.; Kotaki,H.; Isozaki,S.; Saitoh,Y. Quantitative determination of propranolol in plasma and plasma water from normal subjects and patients with angina pectoris by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 311–319.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 0.5% ascorbic acid solution + 1 mL 1 M pH 10 carbonate buffer + 100 μL 0.05 μg/mL 4-methylpropranolol in MeOH + 3 mL diethyl ether, vortex for 1 min, centrifuge at 1500 g at 4° for 10 min. Remove the organic layer and add it to 500 μL 100 mM orthophosphoric acid, vortex for 1 min, centrifuge, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco C18
Mobile phase: MeOH:MeCN:0.1% triethylamine in water 25:25:50, pH adjusted to 2.5 with 1 M orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: F ex 300 em 375

CHROMATOGRAM
Retention time: 5
Internal standard: 4-methylpropranolol (8)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; rat

REFERENCE
Qureshi,S.A.; Buttar,H.S. High-performance liquid chromatographic determination of propranolol and its metabolites in rat serum, *J.Chromatogr.*, **1988**, *431*, 465–470.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood + 75 ng IS + 6 mL diethyl ether + 2 mL 1 M pH 9.9 sodium carbonate, shake horizontally for 25 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 250 μL 1.2 M triethylamine in dichloromethane, add 200 μL reagent, vortex briefly, let stand at room temperature for 1 h, evaporate under a stream of nitrogen at 30° for 17 min, reconstitute the residue in 2 mL 100 mM NaOH, agitate for 5 min, add 6 mL diethyl ether, shake for 15 min, centrifuge at 2000 g. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, cool the tubes to 0°, reconstitute the residue in 250 μL trifluoroacetic acid, vortex briefly, let stand at 0°, after 7 min add 2 mL 2 M NaOH, add 6 mL diethyl ether. Remove the organic layer and add it to 150 μL 100 mM orthophosphoric acid, extract, inject a 20 μL aliquot of the aqueous phase. (Prepare the reagent by mixing 2 mL 250 mM 1,3-dicyclohexylcarbodiimide in dichloromethane with 1 mmole N-tert-butoxycarbonyl-L-

leucine (Boc-L-Leu) in 3 mL dichloromethane, vortex briefly, react at 0° for 75 min, filter, use the filtrate as the reagent. Store at 0°.)

HPLC VARIABLES
Guard column: 30 × 2.1 5 μm Spheri-5-RP-18
Column: 250 × 2 5 μm Ultrasphere ODS
Mobile phase: MeOH:20 mM pH 2.8 $(NH_4)H_2PO_4$ 72:28
Flow rate: 0.2
Injection volume: 20
Detector: F ex 228 em 290 (cutoff filter)

CHROMATOGRAM
Retention time: 18 (S-(-)), 29 (S-(+))
Internal standard: cyclopentyldesisopropylpropranolol (Pierce) (33, 54 (enantiomers))
Limit of detection: 2.5 ng/mL

KEY WORDS
derivatization; chiral; rat; whole blood; pharmacokinetics

REFERENCE
Guttendorf,R.J.; Kostenbauder,H.B.; Wedlund,P.J. Quantification of propranolol enantiomers in small blood samples from rats by reversed-phase high-performance liquid chromatography after chiral derivatization, *J.Chromatogr.*, **1989**, *489*, 333–343.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 600 ng/mL IS in water + 200 μL 10% sodium bicarbonate + 5 mL diethyl ether, shake at 20 rpm for 15 min, centrifuge at 400 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Add 50 μL 5 μL/mL R(+)-phenylethylisocyanate in diethyl ether to the residue, vortex vigorously for 30 s, keep at 4° for 30 min, allow to warm to room temperature, vortex for 15 s, evaporate under nitrogen, reconstitute in 100 μL mobile phase, let stand at room temperature for 20 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 25-37 μm Whatman Co:Pell ODS pellicular C18
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeOH:isopropanol:dichloromethane:water 67:7.5:1:25.5
Flow rate: 0.7
Injection volume: 20
Detector: F ex 220 em 300 (cut-off filter)

CHROMATOGRAM
Retention time: 13.7 (S(-)), 14.9 (R(+))
Internal standard: (±)-N-cyclopentyldesisopropylpropranolol (19.3, 21.2)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; human; rat; chiral; derivatization

REFERENCE
Laganière,S.; Kwong,E.; Shen,D.D. Stereoselective high-performance liquid chromatographic assay for propranolol enantiomers in serum, *J.Chromatogr.*, **1989**, *488*, 407–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.5 N pH 10 sodium bicarbonate/sodium carbonate buffer + 100 μL 300 ng/mL IS, vortex for 10 s, add to a 150 × 14 column packed with a 45

mm layer of Extrelut on top of a 25 mm layer of anhydrous sodium sulfate. elute with 15 mL diethyl ether. Add the eluate to 100 μL 10 mM trichloroacetic acid in dry dichloromethane, add 100 μL 250 mM (R,R)-O,O-diacetyltartaric acid anhydride in acetic acid:dichloromethane 20:80, mix, heat at 40° for 4 h, evaporate to dryness under a stream of nitrogen, wash the tube down with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 20 μL acetic acid, add 20 μL MeOH, add 60 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4 10 μm Lichrosorb RP18
Column: 125 × 4 5 μm C18 Hypersil
Mobile phase: MeCN:2% aqueous acetic acid 70:30, adjusted to pH 4.0 with concentrated ammonia
Flow rate: 1
Injection volume: 50
Detector: F ex 290 em 335

CHROMATOGRAM
Retention time: 4 (R), 6 (S)
Internal standard: N-tert-butylpropranolol (Synthesize as follows. Reflux 2.9 g 1-naphthol, 30 mL epichlorohydrin (Caution! Epichlorohydrin is a carcinogen!), and 4.4 g (ca. 22 mequiv OH^-, Merck) ion-exchange resin for 4 h, filter, evaporate to dryness, take the residue up in toluene, evaporate to dryness, take the residue up in toluene, evaporate to dryness, take up the residue in hot petroleum ether, evaporate to dryness. Reflux the residue with 30 mL tert-butylamine for 16 h, evaporate to dryness, take up the residue in 30 mL diethyl ether, wash with two 15 mL portions of water, add 4.5 mL 4 M HCl. Remove the organic phase and the product crystallizes after several h, recrystallize from water to give N-tert-butylpropranolol hydrochloride (mp 180°).) (5 (R), 7 (S))
Limit of detection: 0.5 ng/mL (R), 1 ng/mL (S)

KEY WORDS
derivatization; plasma; chiral; pharmacokinetics

REFERENCE
Lindner,W.; Rath,M.; Stoschitzky,K.; Uray,G. Enantioselective drug monitoring of (*R*)- and (*S*)-propranolol in human plasma via derivatization with optically active (*R,R*)-O,O-diacetyl tartaric acid anhydride, *J.Chromatogr.*, **1989**, *487*, 375–383.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL pH 10 boric acid/KCl buffer + 5 mL toluene, shake on a mechanical shaker for 30 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute with 50 μL MeOH:triethylamine 99:1, add 20 μL 0.1% NAPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (NAPIC is (-)-(S)-naproxen isocyanate; synthesis is as follows (protect from light). Dissolve 1 g (+)-(S)-naproxen in 30 mL acetone, cool to 0°, add a solution of 700 μL triethylamine in 2 mL acetone dropwise, add a solution of 450 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 310 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain NAPIC as an oil that crystallized in the desiccator (mp 48°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: F ex 276 em 356

CHROMATOGRAM
Retention time: 18.6 (R), 20.3 (S)

KEY WORDS
derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 200 mM pH 10.5 phosphate buffer + 5 mg ascorbic acid + 4 mL ethyl acetate, shake vigorously for 10 min, centrifuge at 1500 g for 15 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL chloroform, add 30 μL triethylamine, add 2 μL R-(+)-phenylethylisocyanate, shake, let stand at room temperature for 30 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeOH:water 60.5:39.5
Flow rate: 1.2
Injection volume: 50
Detector: F ex 228 em 340 (cutoff filter)

CHROMATOGRAM
Retention time: 27 (-), 31 (+)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Schaefer,H.G.; Spahn,H.; Lopez,L.M.; Derendorf,H. Simultaneous determination of propranolol and 4-hydroxypropranolol enantiomers after chiral derivatization using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 351–359.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C18 Bond Elut SPE cartridge with 1. mL MeOH and 1.5 mL water. 1 mL Whole blood + 1 mL water + 100 μL 400 ng/mL methyl 4-propranolol hydrochloride in water + 3 mL 1 M sodium carbonate + 10 mL heptane:isopropanol 98:2, shake at 80-100 strokes/min for 15 min, centrifuge at 1500 g at 4° for 5 min. Remove 7 mL of the organic phase and evaporate it to dryness at 50° under a stream of nitrogen. Take up the residue in 150 μL acetone, vortex for 30 s, add 100 μL pH 7.85 borate buffer, add 50 μL (+)-1-(9-fluorenyl)ethyl chloroformate 500 μg/mL in acetone, mix for 30 s, let stand for 5 min at room temperature. Add 800 μL water and the reaction mixture to the SPE cartridge, wash with 1.5 mL isooctane, elute with 500 μL dichloromethane. Evaporate the eluate to dryness and take up the residue in 35 μL MeCN, vortex vigorously for 15 s, add 75 μL water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 Nova-Pak C18 radial compression
Mobile phase: MeCN:water 75:25
Flow rate: 2
Injection volume: 100

Detector: F ex 260 em 340

CHROMATOGRAM
Retention time: 10.5 (S(-)), 11.3 (R(+))
Internal standard: methyl 4-propranolol hydrochloride (14.5 (S), 15.5 (R))
Limit of quantitation: 0.5 ng/mL

KEY WORDS
whole blood; chiral; derivatization; SPE

REFERENCE
Roux,A.; Blanchot,G.; Baglin,A.; Flouvat,B. Liquid chromatographic analysis of propranolol enantiomers in human blood using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate, *J.Chromatogr.*, **1991**, *570*, 453–461.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 ng (+)-bufuralol + 4 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, wash with 5 mL EtOH:water 30:70, elute with 8 mL EtOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL 2 mg/mL (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide in MeCN containing 0.01% quinuclidine, heat at 60° for 20 min, add 50 μL MeOH, evaporate to dryness, reconstitute with 1 mL EtOH:water 90:10, add to an 18 × 6 column containing 100 mg carboxymethyl Sephadex LH-20, wash with EtOH:water 90:10 at 0.2 mL/min, elute with 3 mL 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute the residue in 50-100 μL mobile phase, inject a 10-20 μL aliquot. (Synthesis of (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide is as follows. Reflux 210 g 1-bromo-2-methylnaphthalene, 160 g N-bromosuccinimide, 1 g benzoyl peroxide, and 250 mL carbon tetrachloride for 2.5 h, add 250 mL carbon tetrachloride, filter while warm, wash the residue several times with solvent. Concentrate and cool the filtrate to give 1-bromo-2-bromomethylnaphthalene (mp 230-240°) (J. Org. Chem. 1949, 14, 375). Dissolve 90 g 1-bromo-2-bromomethylnaphthalene in 400 mL chloroform, reflux, add 46.5 g powdered hexamine in portions, remove the hexaminium salt by filtration. Reflux this salt in 650 mL 50% acetic acid for 1 h, add 105 mL concentrated HCl, reflux for 5 min, cool, obtain 1-bromo-2-naphthaldehyde (mp 119-120°) by filtration. Heat 11 g 1-bromo-2-naphthaldehyde in 275 mL acetone at 60-68°, add a hot solution of 14 g potassium permanganate in 330 mL water over 30 min, heat for another 30 min, pass in sulfur dioxide (sodium metabisulfite ?) until the solution is clear, pour into water to give 1-bromo-2-naphthoic acid, purify by forming the ammonium salt and reprecipitating. Reflux 1-bromo-2-naphthoic acid in MeOH in the presence of sulfuric acid to give methyl 1-bromo-2-naphthoate. Heat methyl-1-bromo-2-naphthoate with copper bronze at 270-280° for 20 min, while still hot extract with toluene, cool to obtain dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate, obtain more crystals by evaporating some of the solvent, recrystallize from EtOH to give dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate (mp 158°) (J. Chem. Soc. 1955, 1242). Add 8 g lithium tri-tert-butoxyaluminohydride in portions to 2.8 g dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate in 150 mL anhydrous benzene:ether 50:50 (Caution! Benzene is a carcinogen!), heat at 80° for 2 h, acidify with 5% HCl. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness, chromatograph on 50 g silica gel with hexane:ethyl acetate 80:20, recrystallize the product from hexane/acetone to give methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate (mp 117.5-118.5°). Add 5 mL 30% hydrogen bromide in acetic acid to 2 g methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL acetic acid, stir at 50° for 10 min, pour into ice-water, filter, chromatograph the solid on 40 g silica gel with hexane:ethyl acetate 30:1 to give methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate as pale yellow needles (mp 137-138°). Add 400 mg sodium borohydride to 1.9 g methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL DMSO, stir at 60° for 15 min, pour into ice-water, acidify with concentrated HCl, chromatograph the crude product on 40 g silica gel with hexane:ethyl acetate 10:1, recrystallize from MeOH to give methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate as colorless needles mp 97-98°. Add 30 mL 10% KOH to 1.2 g methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate in 50 mL MeOH, reflux for 3 h, pour into ice-water, filter, recrystallize from hexane/ethyl acetate to give 2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 232-233°). Add 4.1 g (-)-brucine in 20 mL EtOH to 3.3 g 2-methyl-1,1'-binaphthalene-2'-carboxylic acid dissolved in 60 mL EtOH, allow to stand overnight, filter, recrystallize the precipitate several times from EtOH. Add 5% HCl to the salt and extract with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness, recrystallize from hexane/acetone to give (-)-2-methyl-1,1'-binaphthalene-2'-carbox-

ylic acid as colorless needles (mp 229-229.5°; $[\alpha]_D^{20}$ -41.3° (c = 0.58 in chloroform). Add 3 mL oxalyl chloride to 500 mg (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid in 30 mL anhydrous dichloromethane, stir at room temperature for 2 h, evaporate to give an oily residue, take up in 10 mL dichloromethane, add 2 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, chromatograph on 5 g silica gel with hexane to give (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide as a yellow oil ($[\alpha]_D^{20}$ -42.8° (c = 1.05 in chloroform) (Anal. Sci. 1990, 6, 261).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm spherical silica (Waters)
Mobile phase: Hexane:ethyl acetate:MeOH 90:6:1.8
Injection volume: 10-20
Detector: F ex 318 em 408

CHROMATOGRAM
Retention time: 9 (-), 11 (+)
Internal standard: (+)-bufuralol (5)
Limit of detection: 100 pg

KEY WORDS
derivatization; plasma; SPE; chiral; normal phase

REFERENCE
Shao,G.; Goto,J.; Nambara,T. Separation and determination of propranolol enantiomers in plasma by high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1991**, *14*, 753–763.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL pH 10 boric acid/KCl buffer + 500 mg NaCl + 5 mL toluene, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL dichloromethane, add 50 μL 1% triethylamine in MeOH, add 20 μL 0.1% FLOPIC in dichloromethane, mix, let stand at room temperature for 30 min, add 50 μL 1% ethanolamine in MeOH, mix, let stand at room temperature for 15 min, evaporate to dryness, reconstitute with 1% acetic acid in mobile phase, inject an aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure (Chirality 1989, 1, 223).)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water 75:25
Flow rate: 1
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 18, 20 (enantiomers)
Internal standard: pronethalol (15, 17 (enantiomers))
Limit of detection: 1-2 ng/mL

KEY WORDS
derivatization; plasma; chiral; comparison with other derivatization reagents

REFERENCE
Spahn-Langguth,H.; Podkowik,B.; Stahl,E.; Martin,E.; Mutschler,E. Improved enantiospecific RP-HPLC assays for propranolol in plasma and urine with pronethalol as internal standard, *J.Anal.Toxicol.*, **1991**, *15*, 209–213.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg RP 18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 1 mL pH 9 borate buffer, add to SPE cartridge, wash with 5 mL pH 3.15 phosphate buffer, wash with 3 mL water, wash with 500 µL MeCN:water:phosphate buffer 62:32:6, elute with 1 mL MeCN:water:phosphate buffer 62:32:6, inject a 100 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Spherisorb ODS
Mobile phase: MeCN:water:phosphate buffer 62:32:6
Flow rate: 1
Injection volume: 100
Detector: F ex 290 em 350

CHROMATOGRAM
Retention time: 12
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: celiprolol

KEY WORDS
plasma; SPE; propranolol is IS

REFERENCE
Rostock,G.; Günzel,R.; Glöckl,D. Solid-phase extraction and direct high-performance liquid chromatographic determination of celiprolol in plasma, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1992**, *30*, 512–513.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 µL 1 M pH 10.3 carbonate buffer and 25 µL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 µL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 µL/L orthophosphoric acid + 600 µL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 5.94
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, fluvoxamine, clovoxamine, fenfluramine, amoxapine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 3 μg/mL propranolol hydrochloride in water + 6 mL MTBE, shake 15 min, centrifuge at 1500 g for 15 min. Remove organic layer and add it to 100 μL 0.05 M sulfuric acid, shake 15 min, centrifuge at 1500 g at 4° for 10 min, discard organic layer, inject 50 μL aliquots of aqueous layer.

HPLC VARIABLES
Column: 100 × 2 5 μm ODS Hypersil
Mobile phase: MeCN:10 mM Na_2HPO_4 40:60 containing 40 mM sodium dodecyl sulfate and 3 mM tetrabutylammonium bromide, adjusted to pH 2 with orthophosphoric acid
Flow rate: 0.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: propranolol

OTHER SUBSTANCES
Simultaneous: diltiazem, diltiazem metabolites

KEY WORDS
plasma; microbore; propranolol is IS

REFERENCE
Zoest,A.R.; Hung,C.T.; Wanwimolruk,S. Diltiazem: a sensitive HPLC assay and application to pharmacokinetic study, *J.Liq.Chromatogr.*, **1992**, *15*, 1277–1287.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 1 M NaOH + 5 mL dichloromethane, shake, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL dichloromethane, vortex for 5 s, add 10 μL 0.01% S-(+)-1-(1-naphthyl)ethyl isocyanate, heat at 37° for 2 h, add 20 μL tert-butylamine, evaporate under a stream of nitrogen, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 240 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeOH:THF:200 mM pH 3.6 acetate buffer 51:14:35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: F ex 226 em 333

CHROMATOGRAM
Retention time: 25.2 (R-(+)), 28.3 (S-(-))
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: oxprenolol

KEY WORDS
plasma; chiral; derivatization; propranolol is IS

REFERENCE
Laethem,M.E.; Rosseel,M.T.; Wijnant,P.; Belpaire,F.M. Chiral high-performance liquid chromatographic determination of oxprenolol in plasma, *J.Chromatogr.*, **1993**, *621*, 225–229.

SAMPLE
Matrix: blood
Sample preparation: Inject sample onto column A with mobile phase A and elute for 3 min. Backflush contents of column A onto column B with mobile phase B for 6 min and elute column B with mobile phase B and monitor eluant.

HPLC VARIABLES
Column: A 10 × 3 BioTrap Amine C18 (ChromTech); B 10 × 3 CT-sil C8 guard column + 100 × 4.6 5 μm CT-sil C8 (ChromTech)
Mobile phase: A 48 mM pH 7.0 phosphate buffer; B MeCN:120 mM pH 3.0 phosphate buffer 28:72
Flow rate: A 0.55; B 1
Injection volume: 50
Detector: F ex 220 em 340

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 4.5 ng/mL

KEY WORDS
plasma; column-switching; direct injection

REFERENCE
Hermansson,J.; Grahn,A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase, *J.Chromatogr.A*, **1994**, *660*, 119–129.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 240 μL MeOH:2-aminoheptane 99.7:0.3 at 1.5 mL/min, pass 410 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 250
Detector: F ex 225 em 340

CHROMATOGRAM
Retention time: 14
Limit of detection: 1.3 ng/mL
Limit of quantitation: 4.5 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 2.1
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, promazine, propafenone, propoxyphene, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: dextromethorphan, lidocaine, pentazocine

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 500 μL + NaOH + 3 mL MTBE, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in equal parts of MeCN:triethylamine 99.6:

0.4 and 0.025% 2,3,4,5-tetra-O-acetyl-α-D-glucopyranosyl isothiocyanate in MeCN. Evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:75 mM pH 3 ammonium phosphate 50:50
Flow rate: 1.4
Injection volume: 100
Detector: F ex 216 em 340

CHROMATOGRAM
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; derivatization; chiral; pharmacokinetics

REFERENCE
Bleske,B.E.; Welage,L.S.; Rose,S.; Amidon,G.L.; Shea,M.J. The effect of dosage release formulations on the pharmacokinetics of propranolol stereoisomers in humans, *J.Clin.Pharmacol.*, **1995**, *35*, 374–378.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.4 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge at 13000 rpm for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 SynChropak bulk support (Knauer)
Column: 120 × 4.6 5 μm Spherisorb ODS1 C18
Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)
Flow rate: 1
Detector: UV 232

CHROMATOGRAM
Retention time: 6.2

OTHER SUBSTANCES
Extracted: cyclopropane carboxylic acid ester prodrug

KEY WORDS
plasma

REFERENCE
Hovgaard,L.; Brondsted,H.; Buur,A.; Bundgaard,H. Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents, *Pharm.Res.*, **1995**, *12*, 387–392.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 600 μL 100 mM pH 8.9 borate buffer + 500 mg NaCl + 100 μL 1 mg/mL 4-methylpropranolol + 6 mL hexane:butanol 96:4, shake for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 180 μL 5 mM sulfuric acid, shake for 10 min, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the aqueous layer. Alternatively, to determine unbound propranolol filter (Amicon centricon-30) plasma while centrifuging at 5500 g for 45-50 min. 1.4 mL Ultrafiltrate + 600 μL 100 mM pH 8.9 borate buffer + 500 mg NaCl + 100 μL 1 mg/mL 4-methylpropranolol + 6 mL hexane:butanol 96:4, shake for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 150 μL 5 mM sulfuric acid, shake for 10 min, centrifuge at 2500 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Permaphase ODS (DuPont)

Column: 110 × 4.7 5 μm Partisil 5 ODS 3
Mobile phase: MeCN:MeOH:40 mM ammonium chloride:triethylamine 20:40:40:0.08 containing 5 mM sodium 1-octanesulfonate, adjusted to pH 6.9 with 85% phosphoric acid
Flow rate: 1.4
Injection volume: 50-100
Detector: F ex 293 em 375

CHROMATOGRAM
Retention time: 3.7
Internal standard: 4-methylpropranolol (5.5)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Panton,L.B.; Guillen,G.J.; Williams,L.; Graves,J.E.; Vivas,C.; Cediel,M.; Pollock,M.L.; Garzarella,L.; Krumerman,J.; Derendorf,H.; Lowenthal,D.T. The lack of effect of aerobic exercise training on propranolol pharmacokinetics in young and elderly adults, *J.Clin.Pharmacol.*, **1995**, *35*, 885–894.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 1 mL MeCN + 25 μL 2 μg/mL pronethalol hydrochloride, vortex for 15 s, centrifuge at 10000 rpm for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50-60°, reconstitute the residue in 100 μL MeOH, vortex briefly, inject a 90 μL aliquot. (Silanize glassware with 5% dimethyldichlorosilane in toluene, rinse with toluene, rinse with MeOH, dry in air.)

HPLC VARIABLES
Guard column: 10 × 4.6 cyano (Alltech)
Column: 250 × 4.6 5 μm Hypersil CN
Mobile phase: MeCN:buffer 35:65 (Buffer was 1% acetic acid containing 0.2% triethylamine, pH 3.6.)
Flow rate: 1.5
Injection volume: 90
Detector: F ex 230 em 340

CHROMATOGRAM
Retention time: 9.5
Internal standard: pronethalol (7.5)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: aspirin, ibuprofen, procainamide, theophylline
Noninterfering: acetaminophen, diltiazem, dipyridamole, furosemide, hydrochlorothiazide, niacin, nifedipine, phenylpropanolamine
Interfering: hydralazine, isosorbide dinitrate, nitroglycerin, quinidine, verapamil

KEY WORDS
serum; plasma; pharmacokinetics

REFERENCE
Rekhi,G.S.; Jambhekar,S.S.; Souney,P.F.; Williams,D.A. A fluorimetric liquid chromatographic method for the determination of propranolol in human serum/plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1499–1505.

SAMPLE
Matrix: blood
Sample preparation: Condition an Styrosorb cross-linked polystyrene (Biochrom, Moscow) or Sep-Pak C18 SPE cartridge with two 3 mL portions of MeOH and two 3 mL portions of water.

Add 1 mL serum containing 2.5 μg metoprolol to the SPE cartridge, wash with two 3 mL portions of water, elute with 600 μL MeOH:diethylamine 99.7:0.3. Evaporate the eluate to dryness under a stream of air at 40°, reconstitute with 50 μL n-heptane:isopropanol:MeOH 83:13:4, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Silasorb-NH2 (Elsico, Moscow)
Mobile phase: n-Heptane:isopropanol:MeOH 83:13:4
Flow rate: 2.5
Injection volume: 20
Detector: F ex 220 em 320 (cut-off filter)

CHROMATOGRAM
Retention time: 3.4
Internal standard: metoprolol (4.1)
Limit of detection: 4 ng/mL

KEY WORDS
SPE: serum; silanize glassware

REFERENCE
Rumiantsev,D.O.; Ivanova,T.V. Solid-phase extraction of Styrosorb cartridges as a sample pretreatment method in the stereoselective analysis of propranolol in human serum, *J.Chromatogr.B*, **1995**, *674*, 301–305.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 100 mM KOH + 30 μL 2 μg/mL (R,S)-n-pentyl propranolol hydrochloride, vortex, add 7 mL n-hexane:n-butanol 99:1, extract. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 20% phosgene in toluene, add 1 mg 4-dimethylaminopyridine, heat at 40° for 3 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 LiChrosorb Si 100 modified with (R,R)-DACH-DNB (see J. Chromatogr. 1991, 539, 25)
Mobile phase: Dichloromethane:MeOH 99.75:0.25
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Internal standard: (R,S)-n-pentyl propranolol hydrochloride
Limit of detection: 0.5-0.6 ng/mL

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Stoschitzky,K.; Kahr,S.; Donnerer,J.; Schumacher,M.; Luha,O.; Maier,R.; Klein,W.; Lindner,W. Stereoselective increase of plasma concentrations of the enantiomers of propranolol and atenolol during exercise, *Clin.Pharmacol.Ther.*, **1995**, *57*, 543–551.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 290

CHROMATOGRAM

Retention time: 6.76
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, microsomal incubations
Sample preparation: Plasma. 100 μL Plasma + 500 μL 1250 U/mL β-glucuronidase/sulfatase (Helix pomatia, Sigma) in 500 mM pH 5.2 sodium acetate buffer, heat at 37° for 18 h. 500 μL Plasma or deconjugated plasma + 100 ng labetalol + 250 μL 1 M pH 10.3 sodium carbonate + 5 mL ether, shake for 10 min on a reciprocating shaker, centrifuge at 200 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, centrifuge for 4 min, inject an aliquot. Microsomal incu-

bations. 100 μL Microsomal incubation + 600 μL labetalol in MeCN at 4°, centrifuge, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak C18 (Radial-Pak)
Mobile phase: MeCN:20 mM phosphoric acid 26:74 containing 2.7 mM dibutylamine
Injection volume: 10-100
Detector: F ex 240 em 350

CHROMATOGRAM
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; liver

REFERENCE
Du Souich,P.; Maurice,H.; Héroux,L. Contribution of the small intestine to the first-pass uptake and systemic clearance of propranolol in rabbits, *Drug Metab.Dispos.*, **1995**, *23*, 279–284.

SAMPLE
Matrix: blood, perfusate
Sample preparation: 1 mL Serum, plasma, or liver perfusate + 100 μL 10% ascorbic acid + 100 μL 1.5 μg/mL 4-methylpropranolol + 2 mL pH 10 1 M sodium carbonate + 5 mL diethyl ether, vortex for 3 min, centrifuge at 1200 g for 10 min. Remove upper organic phase and evaporate to dryness under a stream of nitrogen at room temperature. Reconstitute in 2 mL mobile phase, inject a 100 μL aliquot. (Acidic metabolites can be determined by adding 250 μL 6 M HCl to the lower aqueous phase, inject 250 μL directly. It may be necessary to extract into ether first.)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChroSpher RP-18
Mobile phase: MeCN:MeOH:water 22:33:45 containing 0.033% triethylamine and 0.044% concentrated phosphoric acid, pH 3.2
Flow rate: 1
Injection volume: 100
Detector: F ex 300 em 375 (em 440 from 8 to 11.5 min)

CHROMATOGRAM
Retention time: 8.58
Internal standard: 4-methylpropranolol (12.98)
Limit of detection: 533 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, hydroxypropranolol, desisopropylpropranolol, propranolol glycol

KEY WORDS
serum; plasma; rat; dog

REFERENCE
Semple,H.A.; Xia,F. Simplified high-performance liquid chromatographic method for propranolol and five metabolites in liver perfusate, rat serum and dog plasma, *J.Chromatogr.B*, **1994**, *655*, 293–299.

SAMPLE
Matrix: blood, saliva
Sample preparation: 50 μL Plasma or saliva + 350 μL 1 μM oxprenolol hydrochloride in water + 100 μL 4 M NaOH, sonicate for 10 min, extract with 3 mL ethyl acetate, centrifuge at 3000 rpm for 3 min. Remove the organic layer and wash it with saturated NaCl solution, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, reconstitute the res-

idue in 100 μL MeCN:water:triethylamine 50:50:0.1, add 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject a 50 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to

obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 56:44:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 24, 31 (enantiomers)
Internal standard: oxprenolol
Limit of detection: 25-29 fmole

KEY WORDS

derivatization; chiral; rat; plasma; pharmacokinetics

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 2.15
Internal standard: cianopramine

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 500 μL 1 M NaOH + 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and pass it through a 20 × 4 column filled with glass wool, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 48:52 containing 1 g/L sodium heptanesulfonate (Buffer was 100 mM citric acid-sodium citrate buffer adjusted to pH 2.85 with 1 M HCl.)
Column temperature: 28
Flow rate: 1.7
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 4
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: penbutolol (F ex 278 em 310)

KEY WORDS
plasma; propranolol is IS

REFERENCE
Bernard,N.; Cuisinaud,G.; Sassard,J. Determination of penbutolol and its hydroxylated metabolite in biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 355–361.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 20 μL 25 μg/mL naphthyzine nitrate + 20 μL 10 M KOH + 50 μL 25 mM tetrabutylammonium phosphate + 5 mL ethyl acetate, vortex for 30 s, centrifuge at 500 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° under a stream of air. Reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4 Nucleosil 5 SA
Mobile phase: MeCN:water:diethylamine:orthophosphoric acid 156:300:1.9:1.55
Flow rate: 1.5
Detector: F ex 225 em 350 (cut-off filter)

CHROMATOGRAM
Retention time: 21.5
Internal standard: naphthyzine nitrate (17.8)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum

REFERENCE
Belolipetskaja,V.G.; Piotrovskii,V.K.; Metelitsa,V.I.; Pavlinov,S.A. Ion-exchange high-performance liquid chromatography in drug assay in biological fluids. V. Propranolol and metabolites, *J.Chromatogr.*, **1989**, *491*, 507–512.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 1 mL urine + 50 μL 40 μg/mL pronethalol in MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 0.5 g NaCl (plasma samples only) + 5 mL toluene, shake horizontally for 30 min, centrifuge at 1500 g at 10° for 15 min. Remove 4 mL of the organic layer and evaporate it under reduced pressure. Reconstitute the residue in 200 μL MeOH, add 50 μL 1% triethylamine in MeOH, add 50 μL 2% R-(+)-phenylethylisocyanate in dichloromethane, vortex briefly, heat at 30° for 35 min, evaporate under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water:acetic acid 70:30:0.1
Column temperature: 28
Flow rate: 1.2
Injection volume: 20
Detector: F ex 295 em 345

CHROMATOGRAM
Retention time: 18 (R), 21 (S)
Internal standard: pronethalol (14 (R), 16 (S))
Limit of detection: 1 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Spahn-Langguth,H.; Podkowik,B.; Stahl,E.; Martin,E.; Mutschler,E. Improved enantiospecific RP-HPLC assays for propranolol in plasma and urine with pronethalol as internal standard, *J.Anal.Toxicol.*, **1991**, *15*, 327–331.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 100 μL IS in MeOH + 100 μL 25% ammonium hydroxide + 2 mL MeOH:diethyl ether 10:90, vortex for 1.5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL 5 μL/mL R-(+)-1-phenylethylisocyanate (Fluka) in diethyl ether, vortex for 30 s, let stand at room temperature for 30 min, evaporate to dryness under a stream of nitrogen, add 100 μL mobile phase, vortex for 30 s, centrifuge at 3000 g for 7 min (plasma only), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm C18 (Brownlee)
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeOH:water 72.5:27.5
Flow rate: 1.6
Injection volume: 50
Detector: F ex 232 em 340

CHROMATOGRAM
Retention time: 5.5 (S), 6.2 (R)
Internal standard: 4-methylpropranolol (Cambridge Research Biochemicals) (8.8 (S), 10.1 (R))
Limit of detection: 1 ng/mL

KEY WORDS
derivatization; plasma; chiral; pharmacokinetics

REFERENCE
Pham-Huy,C.; Sahui-Gnassi,A.; Saada,V.; Gramond,J.P.; Galons,H.; Ellouk-Achard,S.; Levresse,V.; Fompeydie,D.; Claude,J.R. Microassay of propranolol enantiomers and conjugates in human plasma and urine by high-performance liquid chromatography after chiral derivatization for pharmacokinetic study, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1189–1198.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 200 μL pH 9.5 carbonate buffer + 600 μL 3 ng/mL 4-methylpropranolol in ethyl acetate, vortex for 1 min, centrifuge at 10000 rpm for 1.5 min. Remove 540 μL of the organic layer and add it to 40 μL pH 2.2 dilute sulfuric acid, vortex for 1 min, centrifuge, inject a 30 μL aliquot of the lower aqueous layer.

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak alkylphenyl
Mobile phase: MeCN:0.06% phosphoric acid 27:73
Flow rate: 1.4
Injection volume: 30
Detector: F ex 205 em 340

CHROMATOGRAM
Retention time: 6.8
Internal standard: 4-methylpropranolol (8.3)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Kim,E.J.; Yoon,W.H.; Lee,W.I.; Kim,O.N.; Lee,M.G. The effect of dehydration on the disposition kinetics of propranolol in rats, *Biopharm.Drug Dispos.*, **1995**, *16*, 251–257.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with MeOH and water. Hydrolyze 900 μL serum with β-glucuronidase (EC 3.2.1.31 type H-1 from Helix pomatia) at 60° for 1 h, add 500 μL (?) MeOH, centrifuge at 2000 g, add the supernatant to the SPE cartridge, wash with 1 mL water, dry under vacuum, elute with 2 mL MeOH:water 90:10, filter, inject an aliquot. Urine. 900 μL Urine + 500 μL MeOH, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm HP C18
Column: 150 × 4.6 5 μm C8P-50 (Asahipak)
Mobile phase: Gradient. MeOH:buffer 30:70 for 4 min, to 45:55 over 6 min, to 50:50 over 2 min, to 60:40 over 2 min, re-equilibrate at initial conditions for 10 min. (Prepare buffer by mixing 100 mM NaH_2PO_4 and 100 mM Na_2HPO_4 to achieve a pH of 7.0 and adding 10 mM N-cetyl-N,N,N-trimethylammonium bromide.)
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, atenolol, metoprolol, oxprenolol

KEY WORDS
serum; comparison with CE; SPE

REFERENCE
Lukkari,P.; Sirén,H. Ion-pair chromatography and micellar electrokinetic capillary chromatography in analyzing β-adrenergic blocking agents from human biological fluids, *J.Chromatogr.A*, **1995**, *717*, 211–217.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 13.06

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix the compound with (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide in MeCN containing 0.1% quinuclidine, heat at 60° for 20 min, take up the reaction mixture in EtOH:water 90:10, add to an 18 × 6 column packed with 100 mg carboxymethyl Sephadex LH-200, elute with 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute with ethyl acetate, inject an aliquot. (Derivatization occurs on the alcohol. Preparation of (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide is as follows. Treat 1-bromo-2-naphthol with sodium hydride in DMF, add iodomethane, stir at room temperature overnight to obtain 1-bromo-2-methoxynaphthalene (mp 85-86°). Add a solution of 37.7 g 1-bromo-2-methylnaphthalene in 200 mL ether over 1 h to a sonicated mixture of 7 g magnesium turnings in 50 mL ether, the mixture should reflux rapidly (Caution! There may be in an induction period!), sonicate for 2 h after addition is complete, add 200 mL benzene (Caution! Benzene is a carcinogen!), add this mixture dropwise to a stirred mixture of 100 mmoles 1-bromo-2-methoxynaphthalene and 655 mg bis(triphenylphosphine)nickel(II) chloride ($NiCl_2(PPh_3)_2$) in 150 mL benzene at room temperature over 1 h, stir at room temperature overnight, reflux for 3 h, remove the ether by distillation through a short Vigreux column, remove the solvent by evaporation under reduced pressure, remove excess 1-bromo-2-methylnaphthalene by heating at 150°/0.1 mm Hg, cool, dissolve the residue in hexane, pass through silica gel, evaporate to dryness, recrystallize from hexane to obtain 1-methoxy-2'-methylbinaphthalene (mp 118-121°). Reflux 10 mmoles 1-methoxy-2'-methyl-binaphthalene, 1.96 g N-bromosuccinimide, and 100 mg benzoyl peroxide in 70 mL carbon tetrachloride for 3 h, filter, evaporate the filtrate to obtain crude 1-bromomethyl-2'-methoxy-binaphthalene. Dissolve the crude 1-bromomethyl-2'-methoxy-binaphthalene in 60 mL DMSO under nitrogen, slowly add a

sodium ethoxide/nitropropane mixture, stir at room temperature for 3 h, stir at 60° for 3 h, pour into 300 mL ice-water, extract with dichloromethane, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to obtain crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde. (Prepare the sodium ethoxide/nitropropane mixture by dissolving 580 mg sodium in 35 mL EtOH, add 3.25 g 2-nitropropane.) Reflux the crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde in 60 mL acetone, add a solution of 2.36 g potassium permanganate in 60 mL hot water dropwise over 1 h, heat for an additional hour, pass sulfur dioxide through the solution until it becomes clear (sodium metabisulfite may work). Filter off the precipitate and dissolve it in 200 mL hot toluene, add a small amount of activated charcoal, filter while hot, concentrate to about a third of the volume, recrystallize from EtOH:water 1:2 to obtain 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (mp 258.5-260°) (Bull. Chem. Soc. Japan 1986, 59, 2044). Reflux 9.15 g racemic 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 55 mL freshly distilled thionyl chloride for 5 h, evaporate under reduced pressure, add a little benzene, evaporate under reduced pressure, repeat the benzene evaporation twice more to obtain 2'-methoxy-1,1'-binaphthalene-2-carbonyl chloride as a brown solid. Dissolve the acid chloride in 70 mL benzene, add dropwise to 12.8 g (-)-menthol in 100 mL benzene containing 1 g 4-dimethylaminopyridine and 5 mL pyridine, stir overnight at room temperature, heat at 70° for 3 h, cool, dilute with benzene, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous magnesium sulfate in the presence of activated charcoal, evaporate to dryness, remove as much menthol as possible by sublimation under vacuum, chromatograph twice on a column of silica gel with toluene to obtain the (aS,R) menthol ester (mp 145-146° from hexane) and the (aR,R) menthol ester (mp 126-129° from hexane) as well as a mixture of diastereomers. Reflux the (aS,R) menthol ester with KOH in aqueous EtOH for 8-10 h to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (Bull. Chem. Soc. Japan 1989, 62, 1528). Add 1.5 mL oxalyl chloride to a solution of (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 10 mL anhydrous benzene, reflux for 10 h, evaporate to dryness under reduced pressure. Take up the residue in 10 mL anhydrous benzene, add 1 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 5 h, evaporate to dryness, recrystallize from hexane/acetone to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide as orange-yellow needles (mp 143-146°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Kyoto)
Mobile phase: Hexane:ethyl acetate:triethylamine 66.6:33.3:0.1
Detector: F ex 330 em 420

CHROMATOGRAM

Retention time: 6 (+), 7.5 (-)
Limit of detection: 100 fmole

KEY WORDS

derivatization; chiral; normal phase

REFERENCE

Goto,J.; Shao,G.; Fukasawa,M.; Nambara,T.; Miyano,S. A chiral axis derivatization reagent for the resolution of β-adrenergic blockers by liquid chromatography with fluorescence detection, *Anal.Sci.*, **1991**, *7*, 645–647.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES

Column: 200 × 4.6 Silica 100 RP 18
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: k' 9.41 (L), k' 11.31 (D)

OTHER SUBSTANCES
Simultaneous: propylhexedrine
Also analyzed: atenolol, methylphenidate, metipranolol, pindolol, propylhexedrine, talinolol

KEY WORDS
derivatization

REFERENCE
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 90:10
Flow rate: 0.5
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 2.65, k' 3.65 (enantiomers)

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45

Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.48, k' 12.17 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2
Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 16.2

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, acebutolol, sotalol, metoprolol, practolol, pindolol, timolol
Interfering: oxprenolol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:water:10 mM tetrabutylammonium hydrogen sulfate:500 mM KH_2PO_4 15:58:17:10, pH 7.0
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: amrinone

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE
Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve 2 mg tablet or capsule in 10 mL pH 10 solution, extract twice with 2 mL ether, combine extracts, filter, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm β-cyclodextrin bonded C18 (Advanced Separation Technologies)
Mobile phase: MeCN:MeOH:acetic acid:triethylamine 95:5:0.3:0.2
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11, 12 (enantiomers)

OTHER SUBSTANCES
Simultaneous: atenolol, metoprolol

KEY WORDS
capsules; tablets; chiral

REFERENCE
Tran,C.D.; Dotlich,M. Enantiomeric separation of β-blockers by high performance liquid chromatography, *J.Chem.Educ.*, **1995**, *72*, 71–73.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 200 μL MeCN to 100 μL microsomal incubation, centrifuge at 3000 g for 5 min, inject a 120 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.2 Kromasil C18
Mobile phase: MeCN:MeOH:water:acetic acid 25:25:50:0.1
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 4.0

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE

Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 μL Microsomal incubation + 50 μL 6% perchloric acid containing 2% ascorbic acid, centrifuge at 7500 g for 5 min. Removal a 400 μL aliquot of the supernatant and add it to 50 μL 50 μg/mL labetalol, mix, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.5 with orthophosphoric acid
Flow rate: 3
Injection volume: 100
Detector: F ex 295 em 360

CHROMATOGRAM

Retention time: 7.2
Internal standard: labetalol (5.3)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

yeast

REFERENCE

Bichara,N.; Ching,M.S.; Blake,C.L.; Ghabrial,H.; Smallwood,R.A. Propranolol hydroxylation and N-desisopropylation by cytochrome P4502D6. Studies using the yeast-expressed enzyme and NADPH/O2 and cumene hydroperoxide-supported reactions, *Drug Metab.Dispos.*, **1996**, *24*, 112–118.

SAMPLE

Matrix: perfusate

Sample preparation: 2 mL Perfusate + 1 mL 100 mM pH 4 phosphate buffer saturated with NaCl, add 6 mL ether, shake for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL isopropanol, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chiralcel OD
Mobile phase: n-Hexane:EtOH:diethylamine 85:15:0.6
Flow rate: 0.6
Injection volume: 20
Detector: F ex 285 em 340

KEY WORDS

chiral

REFERENCE

Ahmed,S.; Imai,T.; Otagiri,M. Stereoselective hydrolysis and penetration of propranolol prodrugs: In vitro evaluation using hairless mouse skin, *J.Pharm.Sci.*, **1995**, *84*, 877–883.

SAMPLE

Matrix: perfusate

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 1 mL Perfusate + 2 mL water, add to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of

nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, centrifuge at 700 g for 5 min, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeOH:50 mM pH 5.8 phosphate buffer 55:45, final pH 5.1
Column temperature: 40
Flow rate: 1
Injection volume: 30
Detector: F ex 280 em 395

CHROMATOGRAM
Retention time: 7.1
Internal standard: propranolol
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: prazosin
Noninterfering: albuterol, alcuronium, aminophylline, atenolol, atropine, betamethasone, bupivacaine, cortisone, dexamethasone, diazepam, diltiazem, hydrocortisone, hyoscine, hyoscine-N-butylbromide, labetalol, lidocaine, methimazole, metoclopramide, norepinephrine, phenobarbital, L-phenylephrine, phenytoin, prednisolone, prednisone, promethazine, propylthiouracil, pyridoxine, ranitidine, verapamil

KEY WORDS
SPE; propranolol is IS

REFERENCE
Fletcher,A.J.; Addison,R.S.; Mortimer,R.H.; Cannell,G.R. Rapid determination of prazosin in perfusion media by HPLC with solid phase extraction, *J.Liq.Chromatogr.*, **1995**, *18*, 2911–2923.

SAMPLE
Matrix: saliva
Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 μL 10 μg/mL alprenolol, add to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES
Guard column: RCSS silica guard-pack (Waters)
Column: 250 × 4.6 Chiralcel OD-H
Mobile phase: n-Hexane:EtOH:diethylamine 91:9:0.1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 320 cut-off filter

CHROMATOGRAM
Retention time: 11 (R), 14 (S)
Internal standard: (S)-alprenolol (6.5)
Limit of detection: 1.33 ng
Limit of quantitation: 3 ng

KEY WORDS
pharmacokinetics; SPE; chiral

REFERENCE
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 3.5 sodium phosphate buffer 60:40
Flow rate: 1
Detector: UV 215

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: μBondapak C18
Column: μBondapak C18
Mobile phase: MeCN:MeOH:water:triethylamine:85% phosphoric acid 33:22:45:0.03:0.04, pH 3.4
Flow rate: 1.0
Detector: UV 290

REFERENCE
Kunta,J.R.; Goskonda,V.R.; Brotherton,H.O.; Khan,M.A.; Reddy,I.K. Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin, *J.Pharm.Sci.*, **1997**, *86*, 1369–1373.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile) (Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:0.5M sodium perchlorate 40:60
Flow rate: 1

CHROMATOGRAM
Retention time: k' 6.90 (α = 1.11)

OTHER SUBSTANCES
Also analyzed: ketamine

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1 (α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Chiralpak AD (A) or Chiralcel OF (B)

Mobile phase: Hexane:EtOH:diethylamine 95:5:0.5 (A) or hexane:2-propanol:diethylamine 92:8:0.5 (B)
Flow rate: 0.8
Detector: UV 254

KEY WORDS
chiral; α = 1.50, Rs = 2.77 for Chiralpak AD; α = 1.35, Rs = 1.75 for Chiralpak OF

REFERENCE
Aboul-Enein,H.Y.; Bakr,S.A. Enantiomeric resolution of propranolol and analogs on two cellulose (Chiralcel OF and OC) and one amylose (Chiralpak AD) chiral stationary phases, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1137–1145.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES
Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM
Retention time: k' 21.96
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, ephedrine, flecainide, methamphetamine

KEY WORDS
derivatization; complexation

REFERENCE
Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 500 μL aliquot of a 20-120 μg/mL solution in chloroform with 5 μL R-(+)-1-phenylethyl isocyanate, let stand at room temperature for 15 min, add 10 mL 100 mM HCl, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm silica (Alltech)
Mobile phase: Chloroform:MeOH 100:1.2
Flow rate: 1
Injection volume: 15
Detector: UV 313